Developmental neurobiology: **Notch is tops in the developing brain**

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Signalling through the cell-surface receptor molecule Notch may regulate oligodendrocyte differentiation, and consequently help determine the timing, and perhaps the pattern, of myelination in the developing vertebrate central nervous system.

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Myelination of axon tracts in the vertebrate central nervous system (CNS) is essential for rapid impulse conduction. The timing of myelination differs between different tracts in a pattern that is consistent within a single species. The molecular mechanisms underlying this striking feature of neural development remain unknown. Myelin is formed by oligodendrocytes within the CNS. Oligodendrocyte progenitor cells (OPCs) arise in the subventricular zone (SVZ) of the developing brain and migrate from this region to populate white matter areas of the brain. Following arrival in the region of axon targets, OPCs withdraw from the cell cycle and differentiate into non-migratory, multi-branched oligodendrocytes, which mature further to ensheathe and myelinate the axons. The bulk of this migration, differentiation and myelination occurs when neuronal pathways are already well established. Consequently, migrating OPCs must navigate — sometimes for considerable distances — around, over and often along the axons they will ultimately myelinate before differentiating at the appropriate time [1].

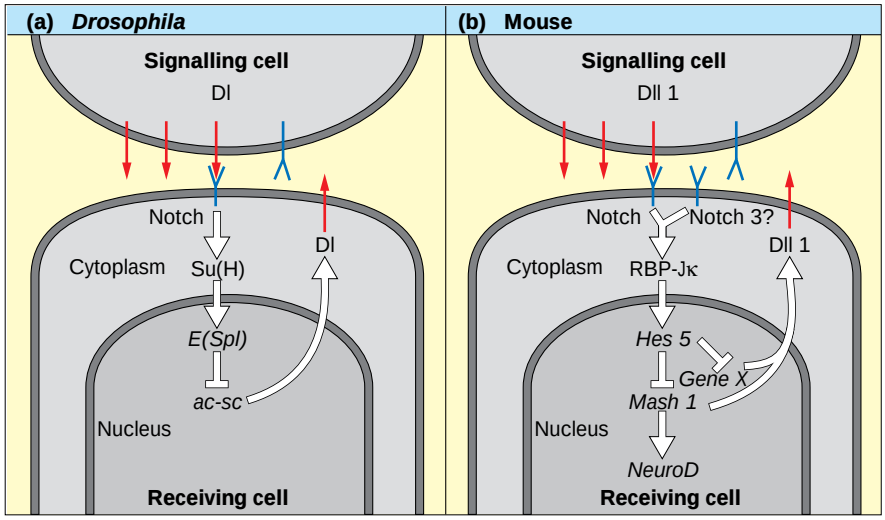
What controls the timing of OPC differentiation and myelination? Cell culture studies show that OPC differentiation is a default pathway which can occur in the absence of any extrinsic signals. Further, there appears to be an internal timing mechanism in the OPCs, such that the daughters of a single OPC grown *in vitro* in the absence of neurons become unresponsive to mitogenic growth factors, withdraw from the cell cycle, and then differentiate more or less synchronously [2]. But if this were the sole mechanism regulating OPC differentiation *in vivo*, myelination of the different regions of the CNS would also occur synchronously, unless OPCs destined for different tracts were born at different times in the SVZ and then followed distinct pathways from this site of common origin. Currently, there is no direct evidence to support this model. An alternative would be that loss of growth factor responsiveness permits differentiation, but that other, local cues instruct the timing in the different tracts. Such a model makes two predictions: first, that local cues should be identifiable; and second, that the cessation of proliferation associated with loss of growth factor responsiveness will not inevitably lead to differentiation, as suggested by the *in vitro* data. Recent evidence suggests that Notch signalling may regulate OPC differentiation in exactly this way [3].

The Notch receptor is a large transmembrane protein which interacts with membrane-bound ligands — Delta or Serrate/Jagged — present on the surfaces of adjacent cells. Ligand binding to Notch activates specific intracellular signalling pathways, which transduce inhibitory signals from the cytoplasm to the nucleus and, as a consequence, repress cellular differentiation (Figure 1) [4–7]. The Notch signalling pathway appears to be well conserved, both structurally and functionally, across species. Signalling through activated Notch is known both to control multiple cell fate determinations (in both invertebrates and vertebrates) and to inhibit developmental processes, such as neurogenesis [8–11] and myogenesis [12–14].

That Notch–ligand interactions can inhibit terminal differentiation is indicated by studies of muscle cell differentiation *in vitro*. For example, Lindsell *et al.* [12] have recently shown that Notch-1-expressing myoblasts fail to differentiate when co-cultured with L cells expressing the Notch ligand Jagged. Activation of Notch by Jagged was found to inhibit the expression of the muscle regulatory gene *myogenin*. Taking a similar approach, Wang *et al.* [3] found that oligodendrocyte differentiation could also be inhibited by Notch–Jagged interactions. OPCs isolated from the rat optic nerve were grown in differentiating medium on a monolayer of Jagged-1-expressing L cells. After three days in culture, a considerable proportion of these cells had failed to differentiate into oligodendrocytes. In contrast, control cultures grown on wild type L cells had nearly all differentiated. Similar effects on differentiation were observed when OPCs were grown in conditioned medium from a cell line engineered to secrete a soluble form of Delta. The Notch pathway could therefore provide the mechanism underlying control of OPC differentiation, with signalling through activated Notch maintaining cells in an undifferentiated state.

Wang *et al.* [3] have also demonstrated that Notch 1 and one of its ligands, Jagged 1, are co-expressed in developing rat optic nerve *in vivo*. Notch 1 is expressed in oligodendroglial cells within the nerve, while Jagged 1 is expressed in retinal ganglion cells and along their axons

Figure 1



The Notch signalling pathways in *Drosophila* and mouse. (a) Inhibition of *Drosophila* neurogenesis through the Notch pathway. A newly-committed neuroblast expresses the Notch ligand Delta (Dl), which signals to an uncommitted neighbouring cell. Notch activation signals via 'Suppressor of Hairless' (Su(H)) to the nucleus, activating *Enhancer of split* (*E(Spl)*) complex genes. *E(Spl)* genes encode repressors of the *achaete-scute* (*ac-sc*) complex, which in turn encode transcription factors required for Dl expression and neurogenesis. The receiving cell thus becomes an epidermoblast rather than a neuroblast. (b) Inhibition of mouse neurogenesis through the Notch pathway. The prospective neuron expresses Delta-like 1 (Dll 1), through which it signals to an adjacent uncommitted neuroepithelial cell. Activated Notch signals via the Su(H) homologue 'Recombination signal sequence Binding Protein for Jκ genes' (RBP-Jκ) to the nucleus, activating *Hes 5*, the vertebrate *E(Spl)* homologue. Hes 5 represses *Mash 1* and '*gene X*' (possibly *neurogenin*), thereby inhibiting expression of both Dll 1 and NeuroD. Mash 1 and NeuroD are transcription factors required for vertebrate neurogenesis, so Notch signalling in the mouse serves both to maintain a population of neuroepithelial cells in a pluripotent state, ready for recruitment as required, and to inhibit neurogenesis. (Adapted from [4].)

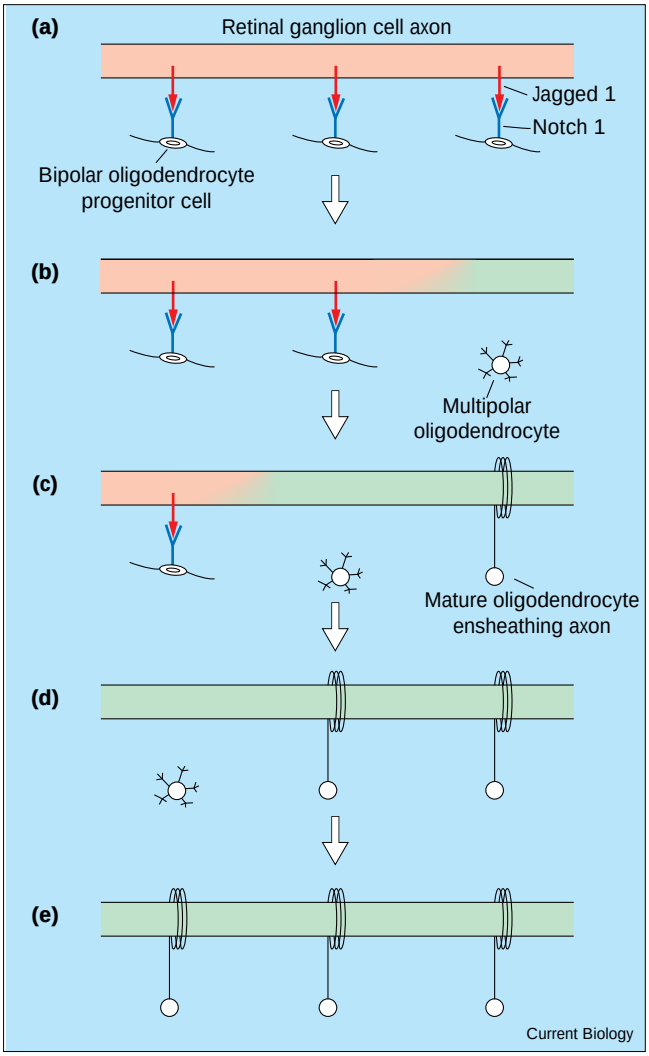
which project in the optic nerve. Levels of expression of Notch 1 and Jagged 1 in these different cell types appear to be coordinately and developmentally down-regulated with a time course that parallels myelination. Consequently, as more and more of the optic nerve becomes myelinated, levels of both Notch 1 and Jagged 1 correspondingly decline. As OPCs are known to migrate along unmyelinated axons in the developing rat optic nerve [1], Notch–Jagged interactions may occur and play a role in preventing premature OPC differentiation. The cell-type-specific distribution of Notch 1 and Jagged 1 is consistent with this hypothesis; OPCs should express Notch 1 on their surfaces if Notch signalling does indeed control OPC differentiation, while Jagged 1 should be expressed by any cells that may potentially interact with OPCs to inhibit their differentiation. Taken together with the *in vitro* data, these observations support a role for Notch signalling in control of the timing of OPC differentiation (Figure 2).

Newly-formed oligodendrocytes isolated from developing rat optic nerve were also found to express Jagged 1 in addition to Notch 1 [3]. This suggests that Jagged 1 expression may be up-regulated as a consequence of OPC differentiation. It is tempting to speculate that a basic-helix–loop–helix protein may regulate expression of Jagged 1 in a similar manner to the regulation of Delta expression by Mash 1/NeuroD during mouse neurogenesis (Figure 1). Regardless of the detailed mechanism, in regions where sufficient oligodendrocytes have been generated, newly-formed oligodendrocytes that express Jagged 1 could signal to neighbouring cells to inhibit further OPC differentiation. A consequence of this inhibition would be to maintain a population of undifferentiated OPCs in the adult CNS after myelination had been completed, just as Notch–ligand interactions are thought to maintain a stem cell population in early neurogenesis [8–11].

Consistent with the hypothesis that a loss of sensitivity to mitogenic factors is permissive for differentiation, rather than instructive, activated Notch appears to inhibit OPC differentiation without stimulating proliferation [3]. Oligodendrocyte progenitor cells grown in the presence of Jagged 1, which inhibits their differentiation, did not incorporate the thymidine analogue BrdU into their nuclei. BrdU uptake could, however, be stimulated in the presence of the mitogen platelet-derived growth factor (PDGF). This is an important observation, as it shows that Notch activation can uncouple proliferation from differentiation and, as a consequence, serve to maintain cells in a state of quiescence (extended G0 phase?) long after the completion of development.

Such quiescent OPCs — as perhaps typified by the observed OPCs present in adult CNS — could be recruited following injury to participate in remyelination. The signals which activate repair might thus concomitantly inactivate the Notch pathway. If so, the efficiency and effectiveness of repair might be dependent on inactivation of the Notch pathway. The demonstration of non-dividing OPCs in human multiple sclerosis lesions [15], in which chronic demyelination is present, suggests that inhibitory signals are present in such lesions which may prevent OPC differentiation into new myelin-forming oligodendrocytes. An attractive hypothesis would be that

Figure 2



The proposed role of the Notch pathway in regulating oligodendrocyte differentiation. Bipolar OPCs, expressing Notch 1 on their surfaces, migrate along the axons of retinal ganglion cells which project in the optic nerve (**a**). These axons express Jagged 1, and Notch–Jagged interactions maintain the OPCs in an immature state, thus preventing premature differentiation. (**b–e**) As Jagged 1 expression is down-regulated in the axon (indicated by the orange-to-green colour change), this inhibitory signal is removed, and the OPCs differentiate into multipolar oligodendrocytes, which then mature further to express myelin and ensheathe the axons. The timing of Jagged expression in relation to oligodendrocyte differentiation and maturation remains to be determined. However, Jagged expression by axons, newly-formed oligodendrocytes and possibly other cells within the optic nerve may not only be important in the timing of oligodendrocyte differentiation and maturation but may play a role in maintaining a pool of OPCs, which could be drawn from as needed during both development and repair.

expression of Notch ligands by reactive astrocytes and other cells within the lesions could inhibit OPC differentiation. Inhibitors of Notch activation may therefore, offer novel therapies for demyelinating disease.

Clearly, signalling through activated Notch could be an important regulator of OPC differentiation in the developing rat optic nerve. Further studies in which Notch activation is manipulated *in vivo* are required to test this hypothesis. It also remains to be established whether the Notch pathway operates ubiquitously within the developing CNS to regulate the timing of OPC differentiation. Certainly, an examination of late myelinating tracts, particularly with respect to the expression of Jagged (and other Notch ligands) and the timing of myelination would be interesting. It might also prove informative to look at myelination in children with Alagille syndrome, where mutations in Notch 1 lead to multiple developmental abnormalities in liver, heart, eye and lung [16,17].

Equally as interesting would be a study of non-myelinated axons, such as those of rat cerebellar granule neurons. Wang *et al.* [3] have predicted that Jagged or other Notch ligands may persist on these axons and continue to inhibit the generation of myelin-forming oligodendrocytes. This would maintain these axons in an unmyelinated state and provide a wider role for Notch–ligand signalling in myelination, determining the timing of myelination and also maintaining myelin patterns in the fully developed CNS. This is just speculation at the moment, but the demonstration that mutations in another Notch, Notch 3, cause the human CNS disease known as 'cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy' (CADASIL) in adults [18] emphasises that Notch signalling is a regulator of cell behaviour throughout life. The role of the Notch pathway in the etiology of diseases of both development and adult life will thus be of interest to clinical and non-clinical neuroscientists alike.

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